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Effects of Bifenthrin on Mycorrhizal Colonization and Growth of Corn

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SUMMARY. The insecticide bifenthrin is a synthetic pyrethroid required by regulation for the production of nursery crops to suppress the red imported fire ant (*Solenopsis invicta*) in Orange and Riverside counties in California. We conducted a greenhouse experiment to analyze the effects of different rates of bifenthrin on the growth and mycorrhizal colonization of 'Silver Queen' corn (*Zea mays*) inoculated with VAM 80®, a mycorrhizal inoculum with spores, hyphae, and root pieces colonized by *Glomus* spp., used to inoculate California native plants in containers. Corn was used because it is the standard indicator plant used for mycorrhizal inoculum potential assays and it is a good host for arbuscular mycorrhizal fungi propagation. The application of bifenthrin had no detrimental effects on mycorrhizal colonization of corn. There were no significant differences in the root length colonized by arbuscules, vesicles, or in the total percentage of mycorrhizal colonization obtained in the plants grown with the different bifenthrin rates 6 weeks after transplanting. However, there were significant interactions on the effects of bifenthrin and mycorrhizal colonization on plant growth. The addition of 12, 15, and 25 ppm of bifenthrin reduced corn biomass of nonmycorrhizal plants, but had no effect on the growth of mycorrhizal plants. There were no significant differences between the mycorrhizal and nonmycorrhizal plants grown with 0, 10, and 12 ppm of bifenthrin. In contrast, inoculation with VAM 80® increased the shoot dry weight of plants grown with 15 and 25 ppm of bifenthrin. This study showed that mycorrhizal colonization can be helpful to overcome some of the negative effects of bifenthrin on the growth of corn.

Mycorrhizal colonization may offer a number of benefits to horticultural crops (Azcón-Aguilar and Barea, 1997;

Larsen et al., 2007). It has been shown that several commercial mycorrhizal inoculants enhance plant growth during nursery container production (Carpio et al., 2003) and improve cuttings' rooting and survival (Druege

et al., 2006). Plants colonized by arbuscular mycorrhizal (AM) fungi are also more efficient in nutrient uptake, more resistant to soil-borne pathogens, and more tolerant to transplanting shock (Azcón-Aguilar and Barea, 1997). However, nursery operations are often subjected to regulations that may affect mycorrhizal colonization, such as the use of certain pesticides (California Department of Food and Agriculture, 2009).

The effects of pesticides on mycorrhizal colonization vary from beneficial to detrimental. While several chemicals inhibit the development of mycorrhizal associations, others do not affect the symbiosis, and the use of certain pesticides even stimulate root colonization by AM fungi and increase their sporulation (Menge, 1982; Trappe et al., 1984). In some cases, contradictory results have been obtained with the same chemical products because the effects of pesticides on mycorrhizal colonization are influenced by the combination of plant species, AM fungi species, pesticide, and dose of application (Sainz et al., 2006; Schweiger and Jakobsen, 1998). Therefore, in horticultural management practices that include the use of AM fungi, it is important to assess the impact of pesticides on the mycorrhizal association.

The insecticide bifenthrin is a synthetic pyrethroid used for the production of nursery crops to suppress the red imported fire ant in quarantined areas of Orange and Riverside counties in California (California Department of Pesticide Regulation, 2009). Contrasting results have been reported on the effects of other synthetic pyrethroids on root colonization by AM fungi. The application of cypermethrin caused significant reduction on the mycorrhizal colonization of peanut (*Arachis hypogaea*), but high concentrations of

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Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
2.54	inch(es)	cm	0.3937
16.3871	inch ³	cm ³	0.0610
0.5933	lb/yard ³	kg·m ⁻³	1.6856
28.3495	oz	g	0.0353
1	ppm	mg·kg ⁻¹	1
(°F - 32) ÷ 1.8	°F	°C	(1.8 × °C) + 32

fenvalerate did not affect the symbiosis and the percentage of mycorrhizal colonization increased when it was applied at concentrations of 5 and 10 ppm (Vijayalakshmi and Rao, 1993). The objective of this study was to determine the effects of bifenthrin on the growth and mycorrhizal colonization of corn inoculated with VAM 80® (Tree of Life Nursery, San Juan Capistrano, CA), a mycorrhizal inoculum that is propagated for large-scale inoculation of California native plants in containers.

Materials and methods

The effects of bifenthrin on plant growth and mycorrhizal colonization were compared in a bioassay conducted with 'Silver Queen' corn, the standard indicator plant used for mycorrhizal inoculum potential assays (Moorman and Reeves, 1979) and a good host for AM fungi propagation. Mycorrhizal and nonmycorrhizal corn plants were grown with several rates of bifenthrin in the greenhouse of Tree of Life Nursery in San Juan Capistrano, CA, from Oct. to Nov. 2002. Average high/low temperatures were 23/4 °C (day/night).

GROWING MEDIUM, BIFENTHRIN RATES, AND MYCORRHIZAL INOCULATION. Corn plants were grown in a mix prepared with 2 calcined clay: 1 silica sand (by volume), one of the growing media used for VAM 80® propagation at Tree of Life Nursery. This medium was mixed in a cement mixer with 2 lb/yard³ of Sierra micro-max® trace element mix (Scotts-Sierra Horticultural Products, Marysville, OH) and 1 lb/yard³ of 18N-2.6P-9.9K Osmocote® controlled-release fertilizer (Scotts Co., Marysville, OH). Five different mixes were prepared with this medium adding no bifenthrin, or 10, 12, 15, and 25 ppm a.i. bifenthrin (5.38, 6.45, 8.07, and 13.45 lb/yard³, respectively, of Talstar nursery granular insecticide; FMC Corp., Philadelphia, PA). These are the rates specified in the Talstar® specimen label to provide protection against imported fire ant for 0 to 6 months, 7 to 12 months, 13 to 24 months, and continuous protection, respectively.

Twenty Deepots™ (40-inch³ plastic containers, 10 inches deep × 2.5 inches diameter; Stuewe and Sons, Corvallis, OR) were filled with

each of the mixes. Ten were left as nonmycorrhizal controls and 10 were inoculated with a layer of VAM 80® placed 6 to 8 cm from the top of the container. VAM 80® consists of spores, hyphae, and root fragments colonized by a species of *Glomus* that sporulates intraradically, and it contains at least 60 propagules/cm³. One 5-d-old pregerminated corn seedling was transplanted into each container. Two support trays with 10 Deepots™ per treatment were randomly distributed in the greenhouse bench and rotated weekly.

HARVESTS. Five randomly selected plants per treatment were harvested 3 and 6 weeks after transplanting. Shoots were separated from roots and were oven-dried at 70 °C and then weighed. Root systems were divided in two parts and the fresh weight was determined on both. A subsample of the root system was maintained fresh and was used to assess the percentage of mycorrhizal colonization. The remaining part was oven-dried and weighed. Total root dry weight was calculated based on fresh-to-dry weight relationships.

Fresh root pieces were cleared and stained using the technique of Koske and Gemma (1989), and 50 1-cm-long root pieces were mounted in polyvinyl alcohol lacto-glycerol on microscope slides. The percentage of mycorrhizal colonization was determined in 100 intersections by the magnified intersection method of McGonigle et al. (1990).

DATA ANALYSIS. Two-way analysis of variance (ANOVA) with bifenthrin rate and mycorrhizal inoculum as factors was performed on plant

growth (root, shoot, total dry mass, and root:shoot ratio). One-way ANOVA was used to analyze significant differences among bifenthrin rates in each inoculum treatment, and differences between mycorrhizal and nonmycorrhizal plants in each bifenthrin rate were analyzed with the Student's *t* test. The percentages of mycorrhizal colonization (by arbuscules, vesicles, and total) were arcsine-square root-transformed and subjected to one-way ANOVA. Mean contrasts were performed using Fisher's protected least significant difference with $P \leq 0.05$ as the level of significance (Zar, 1996).

Results

EFFECTS OF BIFENTHRIN ON MYCORRHIZAL COLONIZATION. Mycorrhizal colonization at the first harvest was too low to be analyzed (data not shown). Six weeks after transplanting, there were no significant differences in the percentage of mycorrhizal colonization of plants grown without bifenthrin or with different rates of bifenthrin (root length colonized by arbuscules, vesicles, and total percentage of mycorrhizal colonization) (Table 1). Corn plants grown with 25 ppm of bifenthrin tended to have a lower percentage of arbuscules than those grown with 0, 10, 12, or 15 ppm, but the difference was not statistically significant (Table 1).

EFFECTS OF BIFENTHRIN ON PLANT GROWTH. There were no significant differences in root, shoot, total dry weight, and root:shoot ratio between mycorrhizal and nonmycorrhizal corn plants grown without

Table 1. Total percentage of mycorrhizal colonization and percentage of arbuscules and vesicles in plants of 'Silver Queen' corn inoculated VAM 80® (mycorrhizal inoculum with spores, hyphae, and root pieces colonized by *Glomus* spp.; Tree of Life Nursery, San Juan Capistrano, CA) 6 weeks after transplanting.

Bifenthrin rate (ppm) ^z	Mycorrhizal colonization	Arbuscules	Vesicles
		mean ± SE (%) ^y	
0	40.2 ± 5.1	9.0 ± 2.3	28.7 ± 5.0
10	38.7 ± 8.8	10.2 ± 2.1	30.7 ± 7.8
12	36.0 ± 6.9	7.5 ± 3.6	29.9 ± 5.8
15	41.3 ± 7.2	8.9 ± 3.3	36.6 ± 7.0
25	35.5 ± 6.3	2.7 ± 1.6	29.7 ± 5.6

^a1 ppm = 1 mg·kg⁻¹.

^bMean ± SE of five replicates. There were no statistically significant differences among the total percentages of mycorrhizal colonization, percentage of arbuscules and vesicles of corn grown without bifenthrin, or with different rates of bifenthrin.

bifenthrin and with the different rates of bifenthrin at the first harvest (data not shown).

At the second harvest, the two-way ANOVA indicated significant interactions in the effect of bifenthrin and mycorrhizal inoculum in corn shoot, root, and total dry weight. The addition of bifenthrin reduced corn biomass of nonmycorrhizal plants but had no effect on the growth of mycorrhizal plants. Nonmycorrhizal corn plants grown with 12, 15, and 25 ppm of bifenthrin had lower shoot, root, and total dry weight than those grown without or with 10 ppm of bifenthrin (Table 2). In contrast, there were no significant differences in the shoot, root, and total dry weight of mycorrhizal plants grown without bifenthrin or with its different rates (Table 2).

There were no significant differences between the dry weight produced by mycorrhizal and nonmycorrhizal plants grown with 0, 10, and 12 ppm of bifenthrin. However, inoculation with VAM 80® increased

the shoot dry weight and total dry weight of the corn plants grown with 15 and 25 ppm of bifenthrin. Mycorrhizal plants grown with 15 and 25 ppm of bifenthrin had higher shoot and total dry weight than nonmycorrhizal plants (Table 2).

Inoculation with VAM 80® decreased biomass allocation to roots. Mycorrhizal plants grown with 0, 10, and 12 ppm of bifenthrin had lower root dry mass than nonmycorrhizal plants (Table 2).

Discussion

The application of bifenthrin had no detrimental effects on mycorrhizal colonization by VAM 80® in corn plants. The total percentage of mycorrhizal colonization ranged from 35% to 41% in the plants grown with different bifenthrin rates, 6 weeks after transplanting. We were particularly interested in the effects of bifenthrin on the percentage of vesicles in roots because these structures and intraradical spores are the main source of infective propagules of this

mycorrhizal inoculum (Klironomos and Hart, 2002). There were no differences between the percentages of vesicles found in plants grown without bifenthrin or with its different rates.

Several studies have shown that the effects of pesticides on mycorrhizal colonization vary with the plant species, the AM fungi species, and the dose of application (Menendez et al., 1999; Spokes et al., 1981). We have previously tested the effects of bifenthrin in different host plants and in different growing media. No significant differences were found in the percentages of mycorrhizal colonization of California sunflower (*Encelia californica*) and white sage (*Salvia apiana*) grown in a nursery mix composed of different barks with different rates of bifenthrin (Corkidi et al., 2002).

More studies are needed to determine what conditions caused plant sensitivity to bifenthrin. The addition of 12, 15, and 25 ppm of bifenthrin reduced the growth of nonmycorrhizal corn plants. The synthetic pyrethroids, fenvalerate and cypermethrin, also reduced the dry weight of peanut (Vijayalakshmi and Rao, 1993), and the application of bifenthrin EC10 caused slight damage to very young leaves of field roses [*Rosa* spp. (Gough, 1990)]. However, Knabke and Hancock (1990) did not find phytotoxic effects of Talstar® 10WP in over 100 different species grown in the field and greenhouse, nor did Spiers et al. (2006) in 'Festival Salmon' gerbera (*Gerbera jamesonii*).

The question remains as to why mycorrhizal colonization increased the dry weight of the mycorrhizal plants grown with 15 and 25 ppm of bifenthrin and helped to overcome the potential negative effects caused by bifenthrin on the growth of nonmycorrhizal plants. Other studies have also shown that mycorrhizal colonization reduced the phytotoxic effects of some pesticides (e.g., Sainz et al., 2006). However, the complex interactions of insecticides and mycorrhizal colonization on plant growth deserve further investigation because insecticides and AM fungi are also known to alter soil microbial communities and plant nutrient availability (Azcón-Aguilar and Barea, 1997; Das and Mukherjee, 2000).

Table 2. Shoot, root, and total dry weight, and root:shoot of mycorrhizal and nonmycorrhizal plants of corn grown with 0, 10, 12, 15, and 25 ppm bifenthrin insecticide 6 weeks after transplanting.

Response variable	Bifenthrin rate (ppm) ^z	Nonmycorrhizal ^y mean ± SE ^x	Mycorrhizal mean ± SE ^x	Mycorrhizal vs. nonmycorrhizal
Shoot dry wt (g) ^z	0	1.00 ± 0.066 a	1.02 ± 0.219 a	NS ^w
	10	1.03 ± 0.080 a	0.87 ± 0.067 a	NS
	12	0.81 ± 0.045 b	0.84 ± 0.105 a	NS
	15	0.81 ± 0.048 b	1.24 ± 0.111 a	**
	25	0.77 ± 0.037 b	1.20 ± 0.087 a	**
Root dry wt (g)	0	0.79 ± 0.063 a	0.48 ± 0.087 a	**
	10	0.73 ± 0.046 ac	0.54 ± 0.026 a	**
	12	0.60 ± 0.041 bc	0.42 ± 0.031 a	**
	15	0.51 ± 0.034 b	0.59 ± 0.027 ab	NS
	25	0.53 ± 0.035 b	0.63 ± 0.047 ab	NS
Total dry wt (g)	0	1.79 ± 0.108 a	1.50 ± 0.306 a	NS
	10	1.76 ± 0.113 a	1.41 ± 0.089 a	*
	12	1.34 ± 0.068 b	1.26 ± 0.131 a	NS
	15	1.32 ± 0.079 b	1.83 ± 0.123 a	*
	25	1.30 ± 0.067 b	1.83 ± 0.134 a	*
Root:shoot (ratio)	0	0.81 ± 0.075 a	0.49 ± 0.019 a	*
	10	0.73 ± 0.054 ac	0.64 ± 0.034 b	NS
	12	0.75 ± 0.057 ac	0.53 ± 0.050 a	*
	15	0.63 ± 0.023 bc	0.49 ± 0.086 a	*
	25	0.70 ± 0.034 ac	0.52 ± 0.008 a	**

^z1 g = 0.0353 oz, 1 ppm = 1 mg·kg⁻¹.

^yMycorrhizal plants were inoculated with VAM 80® (Tree of Life Nursery, San Juan Capistrano, CA), a mycorrhizal inoculum with spores, hyphae, and root pieces colonized by *Glomus* spp.

^xData represent the mean ± SE of five replicates. Means within a column followed by the same letter are not significantly different at $\alpha = 0.05$ as determined by Fisher's protected least significant difference.

^wNS, *, and ** indicate nonsignificant or significant differences between mycorrhizal and nonmycorrhizal plants in each bifenthrin rate (across rows) at $P \leq 0.05$ or $P \leq 0.001$, respectively, according to the Student's *t* test.

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